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Probing Crucial Metabolic Pathways in Fungal Pathogens of Crucifers: Biotransformation of Indole-3-Acetaldoxime, 4-Hydroxyphenylacetaldoxime, and Their Metabolites

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Abstract—Indole-3-acetaldoxime is an intermediate of crucial importance in the biosynthesis of diverse plant secondary metabolites of Cruciferae. The metabolism of indole-3-acetaldoxime to indole-3-acetic acid via indole-3-acetonitrile by fungi that cause important plant diseases in crucifers, *Leptosphaeria maculans* (asexual stage *Phoma lingam*) causative agent of blackleg disease, *Rhizoctonia solani* causative agent of root rot disease, and *Sclerotinia sclerotiorum* causative agent of stem rot disease, is described. As well, the antifungal activity of indole-3-acetaldoxime and metabolites and the synthesis and biotransformation of 4-hydroxyphenylacetaldoxime by the same plant pathogens and by an insect fungal pathogen, *Beauveria bassiana,* are reported.
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Introduction

Indole-3-acetaldoxime (**1**) is an intermediate of crucial importance in the biosynthesis of diverse plant secondary metabolites of Cruciferae. Cruciferous chemical defenses such as indole-3-acetonitrile (**3**), brassinin (**5**), and brassilexin (**6**),[1,2] as well as the indole glucosinolate **2** (glucobrassicin)[3,4] and the plant hormone indole-3-acetic acid (**4**)[5] are derived from (*S*)-tryptophan via acetaldoxime **1** (Scheme 1). Additional acetaldoxime dependent metabolic pathways also operating in crucifers lead to important aryl glucosinolates such as **8** via 4-substituted phenylacetaldoxime (**7**) (Scheme 2). Furthermore, acetaldoximes are also important intermediates in the biosynthesis of cyanogenic glucosides.[4] The various acetaldoxime dependent biosynthetic pathways co-occur in a good number of cruciferous species and aldoxime formation is a step common to amino acid precursors of more than 120 glucosinolates.[3,4] While the role of compounds **5** and **6** as elicited plant defenses, that is, phytoalexins, has been established, the ecological roles of **2**, **3**, **8**, and **9** remain unclear. For example, glucosinolates **2** and **8** (R = H or OH) are constitutive in a great number of crucifers and cause a range of biological effects,[3,4] whereas nitrile **3** appears to show selective antifungal activity and to be inducible in brown mustard (*Brassica juncea*) but constitutive in turnip (*B. rapa*), rapeseed (*B. napus*), and canola (*B. napus*).[2]

Despite producing multiple chemical defenses, crucifers are susceptible to numerous pests and diseases. The susceptibility of crucifers to infection by a wide range of fungi appears to be related to an effective detoxification of their chemical defenses by these phytopathogens.[6,7] Because indole-3-acetaldoxime (**1**) has a pivotal role in the biogenesis of cruciferous defenses, it is most important to establish if common fungal pathogens can metabolize it, as such a process could be highly detrimental to the plant.

Towards this end, we investigated and report here results of the metabolism of **1** by fungi that cause important plant diseases in crucifers: *Leptosphaeria maculans* (Desm.) Ces. et Not. asexual stage *Phoma lingam* (Tode ex Fr.) Desm. causative agent of blackleg disease, *Rhizoctonia solani* Kuhn causative agent of root rot disease, and *Sclerotinia sclerotiorum* (Lib.) de Bary causative agent of stem rot disease. Furthermore, considering the co-occurrence of acetaldoximes **1** and **7a** in cruciferous species, we also investigated the metabolism of **7a** by the same plant pathogens. To determine if these transformations were common to non plant pathogenic fungi, we examined and report the metabolism of **1** and

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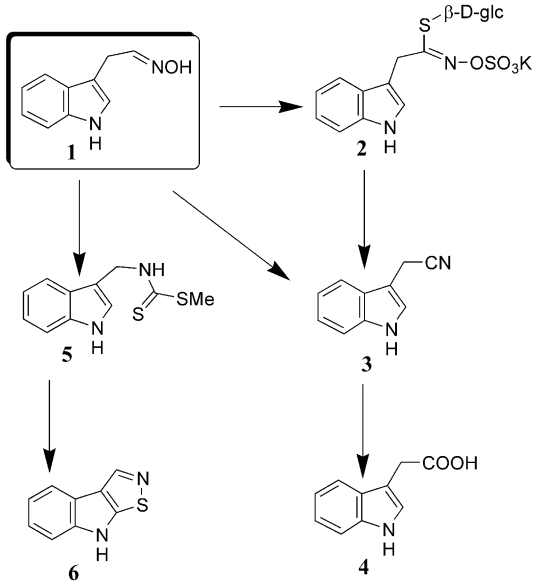
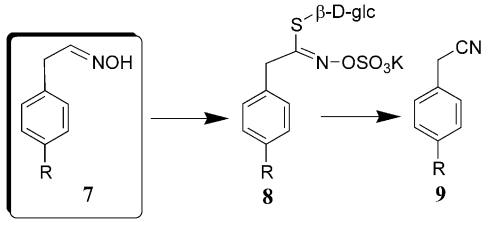


Scheme 1. Metabolism of indole-3-acetaldoxime (**1**) in cruciferous plants.[1–5]



Scheme 2. Metabolism of 4-substituted-phenylacetaldoxime (**7**) in cruciferous plants.[3,4]

7a by a fungus pathogenic to insects, *Beauveria bassiana*. As well, we describe a simple and efficient synthesis of acetaldoxime **7a**, and the antifungal activity of compounds **1**–**3** and **10**. The main objective of this work is to establish if successful cruciferous pathogens can metabolize oxime **1**, the key precursor of tryptophan derived phytoalexins.

Results and Discussion

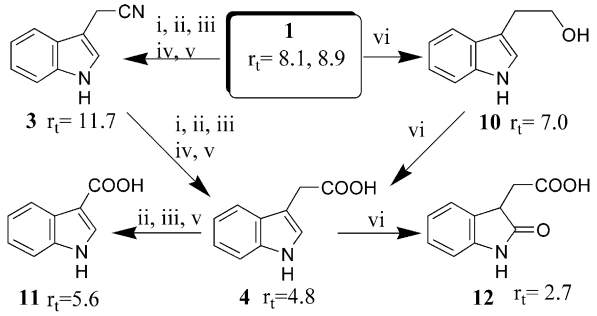
Indole-3-acetaldoxime (**1**) (a mixture of *E*/*Z* isomers)† and glucobrassicin (**2**) were synthesized as previously reported,[2] whereas a new synthesis was devised for 4-hydroxyphenylacetaldoxime (**7a**) (a mixture of *E*/*Z* isomers).† Although acetaldoxime **7a** has been widely used in a great number of biological studies,[3,4] current syntheses of either **7a** or its corresponding aldehyde are rather inefficient. For example, 4-hydroxyphenylacetaldehyde was prepared from synephrine (2-amino-1-(4-hydroxyphenyl)-1-ethanol) following a pinacol-pinacolone type rearrangement in 85% H_3PO_4 in very low yield.[8] Another procedure involved condensation of 4-hydroxybenzaldehyde with [^{14}C]-nitromethane followed by catalytic reduction using platinum and zinc to yield [1-^{14}C]-4-hydroxyphenylacetaldoxime.[9]

†The *E*/*Z* isomers are separable, but in solution both isomers interconverte to equilibrate in a solvent dependent ratio.

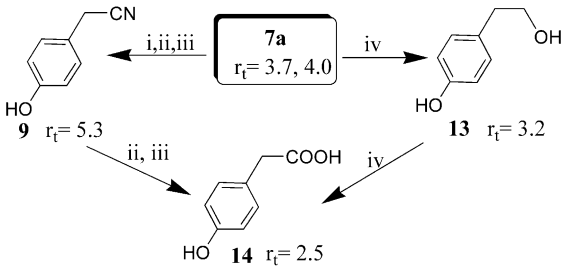
The chemical synthesis of [UL-^{14}C]-4-hydroxyphenylacetaldoxime from tyrosine applied enzymatic conversion of *L*-[UL-^{14}C]tyrosine to 4-hydroxyphenylpyruvic acid, which was allowed to react with hydroxylamine to form 4-hydroxyphenylpyruvic acid oxime, followed by reduction with $NaBH_4$ and decarboxylation to 4-hydroxyphenylacetaldoxime.[10] A more efficient synthesis of **7a** started with oxidation of 2-(4-hydroxyphenyl)-1-ethanol with sulfur trioxide-pyridine complex followed by chromatography and oximation with hydroxylamine hydrochloride.[11] We devised a very simple preparation starting from commercially available 4-hydroxyphenylacetic acid, which was consecutively esterified, reduced with DIBAL to the aldehyde, and the aldehyde allowed to react with hydroxylamine hydrochloride to yield, after column chromatography, a mixture of *E*/*Z* oximes **7a** in good overall yield.

Subsequently, synthetic oximes **1** and **7a** were incubated separately with each fungal isolate (*P. lingam*, *S. sclerotiorum*, *R. solani*, *B. bassiana*), and culture samples were extracted and analyzed over a five day period (all the biotransformation studies were carried out using compounds at a concentration not toxic to these fungi, 10^{-4} M). Because these oximes were not stable in our usual chemically defined fungal culture medium,[7] all biotransformation experiments were carried out in water, as described in the experimental (in the absence of fungi all compounds and intermediates were stable in water for the duration of the experiments; the *E*/*Z* oximes quickly equilibrate in water).

HPLC analysis of the extracts of fungal cultures incubated with **1** (mixture of *E*/*Z* isomers) indicated that both isomers were metabolized by each fungal species to indole-3-acetic acid (**4**), as summarized on Schemes 3 and 4. The structures of all metabolites were established by UV, NMR and MS spectroscopic data and comparison with authentic samples (commercially available). Interestingly, while the insect pathogenic species converted **1** via reduction to tryptophol (**10**, 2-(3-indolyl)-1-ethanol), all plant pathogenic species converted **1** via dehydration to indole-3-acetonitrile (**3**). Similarly, acetaldoxime **7a** (mixture of *E*/*Z* isomers) was converted to 4-hydroxyphenylacetic acid (**14**) via 4-hydroxyphenylacetonitrile



Scheme 3. Metabolism of indole-3-acetaldoxime (**1**) by fungi: (i) *Phoma lingam* BJ-125; (ii) *P. lingam* Laird 2; (iii) *P. lingam* Mayfair 2; (iv) *Sclerotinia sclerotiorum* clone #33; (v) *Rhizoctonia solani* AG 2-1; (vi) *Beauveria bassiana* ATCC 7159; R_t = HPLC retention time in min using mobile phase A (see Experimental).

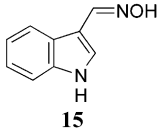


Scheme 4. Metabolism of 4-hydroxyphenylacetaldoxime (**7a**) by fungi: (i) *Phoma lingam* BJ-125; (ii) *Sclerotinia sclerotiorum* clone #33; (iii) *Rhizoctonia solani* AG 2-1; (iv) *Beauveria bassiana* ATCC 7159; R_t = HPLC retention time in min using mobile phase A (see Experimental).

(**9**) by the plant pathogenic fungi, whereas the insect pathogenic fungus carried out similar transformation via 2-(4-hydroxyphenyl)-1-ethanol (**13**). The intermediacy of nitriles **3** and **9** and alcohols **10** and **13** in the fungal transformations of each oxime was demonstrated by separately feeding each of these compounds to fungal cultures and isolating the resulting products. Avirulent isolates of *P. lingam* and *R. solani* further biotransformed indole-3-acetic acid (**4**) to indole-3-carboxylic acid (**11**), whereas *B. bassiana* converted **4** into 2-oxo-indole-3-acetic acid (**12**).

These results are consistent with a previous observation that avirulent isolates of *P. lingam* can further transform indole-3-acetic acid (**4**) to indole-3-carboxylic (**11**).[12] The transformation rates of oximes **1** and **7a** to the respective nitriles **3** and **9** were similar in each species (completed in ca. 24 h), whereas the transformation rates of nitriles **3** and **9** to the respective carboxylic acids **4** and **14** were substantially different. While *S. sclerotiorum* converted nitriles **3** and **9** in ca. 24 h, transformations by *P. lingam* were achieved in ca. 48 h, but *R. solani* converted only 75% of each nitrile in five days (% determined by HPLC). Because in plants indole-3-acetonitrile (**3**) is partly produced from glucosinolate **2**,[4] we also analyzed if similar pathway occurred in *P. lingam*. The transformation of **2** proceeded at relatively slower rates than oxime **1** and lead to no detectable products (ca. 55% of **2** remained untransformed after incubation for five days). That is, the transformation did not appear to proceed via oxime, nitrile, or acid since these compounds were not detected over a five day period.

Contrary to our previous findings on the metabolism of phytoalexins by phytopathogens, where transformation pathways appeared characteristic of a particular phytopathogenic species, the results described above indicate that the metabolism of indole-3-acetaldoxime (**1**) is similar in all plant pathogenic species. Since both acetaldoximes **1** and **7a** were converted to the corresponding nitriles by all plant pathogens, we suspected that the metabolizing enzymes might not be substrate specific. Thus, to further probe the specificity of the fungal enzyme(s) involved in the conversion of oxime **1**, indole-3-carboxaldehyde oxime (**15**) was incubated with *P. lingam* and cultures analyzed by HPLC. Results of these analyses indicated that **15** was transformed (ca. 48–56 h) but this transformation did not appear to occur via the potential intermediates aldehyde, nitrile, or alcohol, since no indole containing products were detected in the cultures.



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To determine if the biotransformations observed were a detoxification process, the antifungal activity of indole-3-acetaldoxime (**1**) and indole-3-acetonitrile (**3**) were determined utilizing radial mycelial growth inhibition assays as described in the experimental. The results on the Table 1 showed that indole-3-acetaldoxime (**1**) was less inhibitory to *P. lingam* BJ-125 (54% at 5.0×10^{-4} M) than indole-3-acetonitrile (70% at 5.0×10^{-4} M). Similarly, oxime **1** was also less toxic to *R. solani* (57% at 5.0×10^{-4} M) than nitrile **3** (71% at 5.0×10^{-4} M). By contrast, oxime **1** was more inhibitory to *S. sclerotiorum* (42% of inhibition at 5.0×10^{-4} M after 24 h) than the nitrile (no effect) but showed lower inhibitory effect on *S. sclerotiorum* than on *P. lingam* and *R. solani*. Indole-3-acetic acid (**4**) had no substantial inhibitory effect on any of the species at the concentrations tested. Neither indole-3-acetaldoxime (**1**) nor indole-3-ethanol (**10**) showed inhibitory activity against *B. bassiana*. Overall, the metabolism of indole-3-acetaldoxime (**1**) lead to detoxification. However, glucosinolate **2** showed no inhibition against *P. lingam*, thus its metabolism did not amount to detoxification.

In summary, the overall results of these biotransformation studies show that all the species were able to metabolize oxime **1**. The plant pathogens, independently of the species, metabolized **1** via nitrile **3**, one of the intermediates also formed in plants. The enzymes responsible for this transformation might be specific to crucifers and to their pathogens, specially considering that the biotransformation of **1** by a non phytopathogen (*B. bassiana*) yielded indole-3-acetic acid via a different intermediate (**10**, tryptophol).

The metabolism of indole-3-acetaldoxime (**1**) has been widely studied in tissues of plants representing diverse families, including Cruciferae.[3–5] It appears that the ability to transform indole-3-acetaldoxime (**1**) to indole-3-acetic acid (**4**)[3–5] and tryptophol (**10**)[13] is widespread. In a previous study, we also reported that tryptophol (**10**) was a metabolite of indole-3-acetaldoxime (**1**) in turnip roots.[1,2] Nonetheless, to the best of our knowledge this is the first study demonstrating that the crucial intermediate oxime **1** is metabolized in both crucifers and their pathogens via similar pathways.

Due to the central biogenetic role played by intermediate **1**, it would be of great interest to establish if during fungal infection of plants, **1** is metabolized and if so, could such a process be inhibited without interfering with the normal plant development. In addition, investigation of the fungal enzymes involved in these transformations, and comparison with related and currently known plant enzymes, should provide a better understanding of their evolution. Furthermore, considering

Table 1. Antifungal activity of indole-3-acetaldehyde oxime (**1**), glucobrassicin (**2**), indole-3-acetonitrile (**3**) and indole-3-ethanol (**10**)

Compd	Concentration (M)	*P. lingam*[a] (% inhibition)[b]	*R. solani*[c] (% inhibition)[b]	*S. sclerotiorum*[d] (% inhibition)[b]	*B. bassiana*[e] (% inhibition)[b]
Indole-3-acetaldoxime (**1**)	5.0×10^{-4}	54±11%	57±4%	42±5%	N.I.[f]
	2.5×10^{-4}	27±11%	N.I.[f]	N.I.[f]	N.I.[f]
	10^{-4}	6±4%	N.I.[f]	N.I.[f]	N.I.[f]
Indole-3-acetonitrile (**3**)	5.0×10^{-4}	70±7%	71±9%	N.I.[f]	-
	2.5×10^{-4}	22±6%	N.I.[f]	N.I.[f]	–
	10^{-4}	N.I.[f]	N.I.[f]	N.I.[f]	–
Indole-3-ethanol (**10**)	5.0×10^{-4}	—	—	—	N.I.[f]
	2.5×10^{-4}	—	—	—	N.I.[f]
	10^{-4}	—	—	—	N.I.[f]
Glucobrassicin (**2**)	5.0×10^{-4}	N.I.[f]	—	—	–
	2.5×10^{-4}	N.I.[f]	—	—	–
	10^{-4}	N.I.[f]	—	—	–

[a]BJ-125, 90 h incubation.
[b]The % of inhibition was calculated using the formula: 100−[(growth on treated/growth in control)×100]+standard deviation; results are the mean of at least three independent experiments.
[c]AG2-1, 72 h incubation.
[d]Clone # 33, 24 h incubation.
[e]ATCC 7159, 120 h incubation.
[f]N.I.: No inhibition (growth on control medium and on medium containing compound is similar).

that a number of secondary metabolic steps[12,14] co-occur in cruciferous pathogens and their hosts, these systems provide an ideal opportunity to investigate the co-evolution of secondary metabolism in plants and their pathogens.

In conclusion, three important cruciferous pathogens and an insect pathogen metabolized indolyl-3-acetaldoxime (**1**) to the corresponding carboxylic acid **4**; however, while the plant pathogen transformed **1** via indolyl-3-acetonitrile (**3**), transformation of **1** by the insect pathogen occurred via tryptophol (**10**).

Experimental

General experimental procedures

All chemicals were purchased from Sigma-Aldrich Canada Ltd., Oakville, ON, except for 4-hydroxyphenylacetonitrile purchased from Alfa Aeser, Ward Hill, MA, USA. All solvents were HPLC grade and used as such, except for CH_2Cl_2 and $CHCl_3$ which were redistilled. Remaining conditions as previously reported.[15]

HPLC analysis was carried out with a high performance liquid chromatograph equipped with quaternary pump, automatic injector, and diode array detector (wavelength range 190–600 nm), degasser, and a Hypersil ODS column (5 μm particle size silica, 4.6 i.d. ×200 mm), equipped with an in-line filter. Mobile phase A: 75% H_2O–25% CH_3CN to 100% CH_3CN, for 35 min, linear gradient, and a flow rate 1.0 mL/min. Mobile phase B: gradient of 100% H_2O–100% MeOH for 60 min, linear gradient, at a flow rate 1.0 mL/min; both water and methanol contained 0.15% triethylamine and 0.18% formic acid.[16] Compounds were identified by comparison with authentic samples.

Indole-3-acetaldehyde oxime (**1**, R_t=8.1, 8.9 min, mobile phase A),[2] glucobrassicin (**2**, R_t=15.7 min, mobile phase B),[17,18] and oxindole-3-acetic acid (**12**)[12] were prepared as previously reported.

Synthesis of 4-hydroxyphenylacetaldoxime (7a). A solution of 4-hydroxyphenylacetic acid (0.50 g, 3.3 mmol) in MeOH (41 mL) and concd H_2SO_4 (1 mL) was refluxed for 2 h, cooled to room temperature, neutralized with aq $NaHCO_3$ (satd), concentrated, and extracted with EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the methyl ester (0.54 g, 3.25 mmol, 99%) as an yellow oil. DIBAL-H (1.5 M in toluene, 2 mL) cooled to −78 °C was added dropwise to a stirred solution of methyl 4-hydroxyphenylacetate (100 mg, 0.60 mmol) in toluene (6 mL, cooled to −78 °C), under Ar atmosphere.[19] The rate of addition was adjusted to keep the internal temperature at −78 °C, and the reaction mixture was stirred for 5 h at −78 °C. The reaction was quenched by slowly adding cold MeOH (100 μL). To the resulting white emulsion, ice-cold 1 N HCl (5 mL) was added slowly with swirling, the emulsion was allowed to warm up to 0 °C, acidified with ice-cold 1 N HCl (7 mL) and stirred for 2 h. The aqueous mixture was then extracted with EtOAc, the combined organic layers were washed with a saturated aq NaCl solution (75 mL), dried over Na_2SO_4, filtered and concentrated to yield 91.6 mg of a colorless oil. The residue was subjected to flash column chromatography on silica gel (CH_2Cl_2–MeOH, 99:1) to yield 61.8 mg (0.45 mmol, 75% yield from ester) of 4-hydroxyphenylacetaldehyde and 7.6 mg (0.06 mmol, 10% from ester) of 2-(4-hydroxyphenyl)-1-ethanol (**13**). Satisfactory spectroscopic data was obtained for both compounds.

Next, a solution of $NH_2OH{\cdot}HCl$ (162.6 mg, 2.34 mmol) and NaOAc (192 mg, 2.34 mmol) in H_2O (2 mL) was added to a solution of 4-hydroxyphenylacetaldehyde (61.8 mg, 0.45 mmol) in 95% aq EtOH (2 mL). After stirring for 6 h at room temperature, the reaction mixture was concentrated to dryness, the resulting residue was diluted with H_2O (10 mL) and extracted with

EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure (95.6 mg). The residue was fractionated by flash column chromatography on silica gel (CH_2Cl_2–MeOH, 99:1) to afford 4-hydroxyphenylacetaldoxime (66.8 mg, 0.44 mmol, 98% from the aldehyde, overall 73%). The spectroscopic data of 4-hydroxyphenylacetaldoxime (**7a**) were similar to published data[10,11,20–22] (since no ^{13}C NMR or FTIR data have been reported to date, we report here complete spectroscopic characterization). R_f = 0.5 (EtOAc–hexane 3:1), R_t = 3.7 and 4.0 min (mobile phase A); mp 104–107 °C (lit.[20] 110–111 °C); UV (MeOH) λ_{max} (log ε) 200 (4.5), 224 (4.0) and 278 (3.5); FTIR ν_{max} 3373, 3257, 3089, 3029, 2898, 1514, 1443, 1243, 924 and 830 cm^{-1}; HRMS-EI *m*/*z* (% relative abundance): measured: 151.0635 [M^+] (56) (151.0634 calcd for $C_8H_9NO_2$), 133.0527 (58), 107.0493 (100), 94.0416 (13), 77.0399 (27), 57.0538 (11); ^1H NMR (500 MHz, CD_3CN) δ 3.37 (d, *J* = 6.3 Hz, 0.2H), 3.55 (d, *J* = 5.4 Hz, 2H), 6.75 (m, 3.2H), 7.03 (d, *J* = 8.6 Hz, 0.2H), 7.06 (d, *J* = 8.5 Hz, 2H), 7.38 (t, *J* = 6.3 Hz, 0.1H), 8.80 (br s, exchangeable) (the intensities of methylene signals at δ 3.55 and 3.37 indicated that the ratio of *Z*/*E* = 10:1); ^{13}C NMR (125.8 MHz, CD_3CN) δ 31.5 (*Z*), 35.8 (*E*), 116.5, 116.7, 129.5, 129.9, 131.2, 132.2, 151.4, 151.7, 156.8, 157.0.

Fungal cultures

Solid cultures of *P. lingam* isolates BJ-125, Mayfair 2 and Laird 2 were grown on V8 agar media as previously reported.[23] Pycnidiospores of these isolates were collected after 15 days of growth on V8 agar media under constant cool fluorescent light at 23 ± 2 °C.[23] *R. solani*, virulent isolate AG2-1, obtained from the AAFC collection (Saskatoon, SK) was grown on potato dextrose agar (PDA) plates at 23 ± 2 °C, under constant light for five days. *S. sclerotiorum* clone #33 was grown on PDA plates at 20 ± 2 °C, under constant darkness and sclerotia were collected after 15 days of incubation.[7] *B. bassiana* ATCC 7159 was obtained from American Type Culture Collection, Manassas, VA, USA. The fungus was grown on PDA plates at 23 ± 2 °C, under constant light.

Metabolism of compounds 1–4, 7a, 9, 10, and 12–15

Spores of *P. lingam* isolates BJ-125 (virulent), Mayfair 2 (avirulent) and Laird 2 (avirulent) were separately inoculated at a concentration of 10^8 spores per 100 mL of liquid minimal media (15 g of glucose, plus thiamine) in 250 mL Erlenmeyer flasks.[23] Mycelia (5 mm diameter agar plugs) cut from the edge of the solid cultures of *R. solani* and *B. bassiana* and sclerotia of *S. sclerotiorum* were separately inoculated into 100 mL of potato dextrose broth (PDB). Incubations (3 days for Laird 2, 4 days for *B. bassiana*, 5 days for *R. solani* and *S. sclerotiorum*, 6 days for *P. lingam* BJ-125 and Mayfair 2), were carried out on a shaker at 130 rpm at 23 ± 2 °C, under constant light. After incubation, the mycelia were filtered, washed with sterile distilled water and transferred to an other 250 mL Erlenmeyer flask with 100 mL of sterile distilled water. Solutions of compounds **1**, **3**, **4**, **7a**, **9**, **10**, and **12–15** in DMSO (final concentrations 10^{-4} M; final concentration of DMSO in water 0.5% v/v) and **2** in H_2O (final concentration 10^{-4} M) were added. Cultures were incubated in constant light, on a shaker at 130 rpm, at 23 ± 2 °C. Samples (5 mL) were withdrawn at different intervals and were either immediately frozen or extracted with ethyl acetate. The ethyl acetate extracts and the aqueous residues were evaporated to dryness and analyzed by HPLC using mobile phase A, except for experiments with glucosinolate **2** in which HPLC analyses were carried out with both mobile phases A and B. Control flasks containing only H_2O, DMSO and the compound, or the mycelia in H_2O and DMSO were also incubated and analyzed similarly.

Bioassays

The antifungal activity of compounds **1–3** and **10** was investigated using the following mycelial radial growth bioassay. A DMSO solution (final concentration 1%) of the compound to be tested (final concentration of each compound 5.0×10^{-4}, 2.5×10^{-4} and 10^{-4} M, compound **2** was dissolved in H_2O) was added to agar medium at ca. 50 °C (for *P. lingam*, *S. sclerotiorum* and *B. bassiana*) or to PDB (for *R. solani*), mixed quickly and poured onto 12-well plates (1 mL). An agar plug (4 mm diameter) cut from edges of 3-day-old solid cultures of *S. sclerotiorum*, 5-day-old solid cultures of *R. solani* and *B. bassiana* and 7-day-old solid cultures of *P. lingam* BJ-125 was placed upside down on the center of each plate, the plates were sealed with parafilm, and incubated at 23 ± 2 °C under constant light for 24 h for *S. sclerotiorum*, 72 h for *R. solani*, 90 h for *P. lingam* BJ-125 and 120 h for *B. bassiana* ATCC 7159. The diameter of the mycelia (in mm) was then measured and compared with control plates containing only DMSO. Each assay was conducted in triplicate and repeated at least three times.

Acknowledgements

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